Effect of N-Acetyl-l-Cycteine on the Growth and the Antibiotic Resistance of both *Pseudomonas aeurginosa* and *Klebsiella pneumoniae*

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Abstract

In this study, *Pseudomonas aeurginosa* and *Klebsiella pneumoniae* were obtained from patient with burn wound infection in Hilla surgical teaching hospital. The effect of NAC on the growth of *Pseudomonas aeurginosa* and *Klebsiella pneumoniae* bacteria was investigated. It was found that NAC at concentrations ranging from (0.4-1)mg/ml cause an inhibition to *Pseudomonas aeurginosa* and *Klebsiella pneumoniae* growth.

Pseudomonas aeurginosa was resistant to all the antibiotics, while *Klebsiella pneumoniae* was sensitive to kanamycin and streptomycin but resistant to other antibiotics before treatment with NAC. On the other hand, the combination effect of NAC(at concentration 0.01mg/ml) and antibiotics on the bacterial growth was also studied. The results showed that *Klebsiella pneumoniae* was sensitive to kanamycin and streptomycin but resistant to other antibiotics, the zone of inhibition is reduced after addition of NAC compared with the zone without NAC. While *Pseudomonas aeurginosa* entirely resistant to the antibiotics after and before the addition of NAC.

تأثير N-acetyl-l-cycteine على نمو ومقاومة بكتريا *Pseudomonas Klebsiella pneumonia* and *aeurginosa* للمضادات الحياتية

الخلاصة

في هذه الدراسة، تم الحصول على *Pseudomonas aeurginosa* and *Klebsiella pneumoniae* المعزولة من مرضى الحروق والتي تم التزويد بها من مستشفى الحلة الجراحي التعليمي. تمت دراسة تأثير NAC على النمو البكتيري وقد لوحظ بان NAC عند التراكيز التي تتراوح بين (0.4-1) ملغم/مل لها تأثير مثبط على النمو البكتيري لكل من بكتريا *Pseudomonas aeurginosa* وبكتريا *Klebsiella pneumoniae* .

وقد أظهرت بكتريا *Pseudomonas aeurginosa* مقاومة لجميع المضادات الحياتية المستخدمة، بينما *Klebsiella pneumoniae* كانت حساسة لكل من kanamycin and streptomycin عند عدم وجود NAC كذلك تم دراسة التأثير المشترك لل NAC مع المضادات الحياتية على نمو العزلات البكتيرية وقد أظهرت النتائج بان *Klebsiella pneumoniae* حساسة لكل من kanamycin and streptomycin ومقاومة للمضادات الحياتية الأخرى . لوحظ أيضا بان مساحة التثبيط قد أصبحت اصغر بوجود NAC مقارنة مع المساحة التثبيطية بدون NAC بينما أظهرت بكتريا *Pseudomonas aeurginosa* مقاومة تامة للمضادات الحياتية بوجود NAC مما يعني إن للـ NAC تأثير مضاد للمضادات الحياتية.

Introduction

N-acetyl-l-cysteine (NAC) is an antioxidant related to l-cysteine, being its acetyl derivative. NAC, is used routinely in medical treatment of chronic bronchitis, cancer, and paracetamol intoxication [1], it is one of the smallest drug molecules in use and it has antibacterial properties [2]. The molecule is a thiol- containing antioxidant that disrupt disulfide bond

in mucus [3, 4], and competitively (cysteine) utilization [5, 6]. Stagnaro, et.al suggests that NAC provides lymphocytic protection against toxic oxygen species[7].

The effect of NAC on bacteria and bacterial biofilms is still relatively unknown, and a better understanding of bacterial responses to NAC may facilitate efficient use of this compound as a biofilm inhibitor.

NAC is able to inhibit growth of both gram positive and gram negative bacteria [8], also NAC decreases the production of extracellular polysaccharide of both gram positive and gram negative bacteria, when it is present in the culture media during growth [9].

This study is aimed to show the effect of NAC on bacterial growth and also its effect on antibiotic effect.

Material and Methods

This study was carried out in Hilla surgical teaching hospital. *Pseudomonas aeurginosa* and *Klebsiella pneumoniae* were obtained from patient with burn wound infection.

1- Effect of NAC on bacterial growth:-

The effect of NAC on bacterial growth was tested by the modified method which was mentioned by [10]:-

1- Nutrient agar was and added in Petri dish then NAC sterilized by filtration) was added to each plate at different volumes to obtain the final concentration of (0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1) mg/ml respectively.

2- The plates were inoculated by bacterial isolates, then incubated for 24hr. at 37Cº.

3- After period of an incubation the results were read according the presence of growth or absent.

2- The Combination effect of some antibiotics with NAC on the growth of isolates:-

Muller Hinton agar is used to show the effect of the following antibiotics Kanamycin, Streptomycin, Gentamycin, Cefixime, Refamicin, and Ciprofloxacin in the presence of 0.01mg/ml of NAC. NAC is sterilized by filtration where as the media is sterilizing by autoclaving at 121Cº for 15min. After solidification of the media, the bacteria was inoculated and spreaded on the culture media and then the antibiotic discs were placed.

Results and Discussion

NAC is used in medical treatment of patient with chronic bronchitis. The positive effects of NAC treatment have primarily been attributed to the mucus-dissolving properties, as well as its ability to decrease biofilm formation which reduce bacterial infection [11].

In table (1), the bacteria used in this study were able to grow in the presence of low concentration of NAC (0.05-0.2)mg/ml, which is the same results obtained by [12]. NAC at concentrations above 0.4mg/ml was able to inhibition the growth of these bacteria.

Table 1 Effect of NAC on bacterial growth

Concentration of NAC mg/ml	Growth of *Pseudomonas aeurginosa*	Growth of *Klebsiella pneumoniae*
0.05	+	+
0.1	+	+
0.2	+	+
0.4	-	-
0.6	-	-
0.8	-	-
1	-	-

(+) growth (-) no growth

NAC, which is one of the most popular mucus liquefying agents, has appreciated in vitro activity against *Pseudomonas aeurginosa* [13] as being growth inhibition. In addition in the presence of NAC, *Klebsiella pneumoniae* was unable to form large colonies, only single and small colonies were present, which changed the texture of the biofilm form [14].

The effects of NAC on inoculum size is a dose dependent it was attributed to a competitively inhibition amino acid (cysteine) utilization [6], or by virtue of possessing a sulfhydryl group, which may react with bacterial cell protein.

Or, on the other hand, NAC is an antioxidant has indirect effect on cell metabolism and extracellular polysaccharide production [11].

Table (2), showed that *Pseudomonas aeuroginosa* was resistance to all the antibiotics without the addition of NAC. While, *Klebsiella pneumoniae* was sensitive to kanamycin and streptomycin but resistance to other antibiotics without addition of NAC.

Table 2 The Combination effect of NAC (at concentration 0.01mg/ml) and antibiotic on bacterial growth

Antibiotics	Without NAC		With NAC	
	Pseudomonas aeurginosa	*Klebsiella pneumoniae*	*Pseudomonas aeurginoa*	*Klebsiella pneumoniae*
Kanamycin	+	-**	+	-*
Streptomycin	+	-**	+	-*
Gentamicin	+	+	+	+
Cefixime	+	+	+	+
Refamicin	+	+	+	+
Ciprofloxacin	+	+	+	+
P value	Less than 0.05		No signifhcant	

(+) Resistance (-) Sensitive
*zone of inhibition≤15
**zone of inhibition>20

Also, this table showed that *Klebsiella pneumoniae* was sensitive to kanamycine and streptomycin but resistance to other antibiotics after the addition of NAC. It was shown previously that NAC diminishes the activity of aminocyclitol antibiotics, neomycin, streptomycin and

kanamycin [15, 16]. It was found that the combination of streptomycin and kanamycin with NAC was antagonistic against *Klebsiella pneumoniae*. The inhibition zone is reduced after the addition of NAC.

It was seen that *Pseudomonas aeurginosa* was not inhibited by the addition of NAC.

NAC is considered to be a nonantibiotic drug but to have antibacterial (bacteriostatic) properties [17] when added to the media alone. It is an effective mucolytic agent having antagonistic effect to the activity to the several antibiotics [18].

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